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LABORATORY EVALUATION OF THE TOXICITY OF PROTEUS, PYMETROZINE, DELTAMETHRIN, AND PIRIMICARB ON LADY BEETLE *HIPPODAMIA VARIEGATA* (GOEZE) (COL.: COCCINELLIDAE)

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Abstract: The implementation of an Integrated Pest Management (IPM) program requires selecting and using chemicals which are least harmful to natural enemies. In this study, the acute toxicity of the recommended field concentration of four conventional insecticides was evaluated in reference to the different life stages (L_3, L_4 and adult) of the variegated lady beetle *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). The evaluated insecticides were Proteus, pymetrozine, deltamethrin, and pirimicarb. A completely randomized design (CRD) with four replications was used as the experimental design. The means were separated by the least significant difference (LSD). The mortalities of those predators treated with the field recommended concentrations of various insecticides, were significantly different. Proteus showed strong toxicity toward the different life stages of the predator. However, pymetrozine and pirimicarb caused less than a 50% mortality. Based on the lethal concentration (LC_{50}) values at 24 h after treatment, the adult predator was the most susceptible to proteus, followed by deltamethrin, pymetrozine, and pirimicarb values of 35.977, 358.757, 915.667, and 2616.113. Based on sublethal concentrations (LC_{30}), these values were 22.718, 261.957, 569.879, and 1521.424. Based on International Organization of Biological Control (IOBC) classification, the insecticides pirimicarb and pymetrozine were both categorized as having a Class 1 toxicity level (harmless), deltamethrin as having a Class 2 level (slightly harmful), and Proteus, a Class 4 toxicity level (harmful).

Key words: acute toxicity, bioassay, *Hippodamia variegata*, insecticide, sublethal

INTRODUCTION

Integrated Pest Management (IPM) relies on a combination of biological, cultural, and chemical tactics that reduce pests to tolerable levels by augmenting natural enemies. Cultural, physical, and chemical methods are used in IPM. Whenever possible, those chemical compounds used in integrated pest control programs must not cause any serious damage to the natural enemies (Oomen 1998; Talebi *et al*. 2008). The implementation of an integrated pest management program requires selecting and using chemicals which not only have an effective pest control, but also cause a low level of harm to natural enemies (Croft 1990).

The lady beetles are a large group containing many important natural enemies. Both larvae and adults are predaceous, feeding primarily on aphids, scale insects, mealy bugs, and whiteflies (Puttaradriah and Basavahna 1953). The variegated lady beetle *Hippodamia variegata* (Goeze) has a large distribution at the Palearctic Ecozone and has since spread to the Nearctic (Obrycki and Cady 1990). It has been reported as the most important natural enemy of aphids infesting various crops. The infested crops include peppers from Bulgaria, corn from the Ukraine, strawberries from Italy, grains in India, and cotton in Turkmenistan (Kontodimas and Stathas 2005). The predator has also been reported in parts of northern, central, and western Iran and seems to be active in other areas (Vojdani 1964; Rajabi 1986).

The variegated lady beetle plays an important role in controlling pests like aphids. As with other natural agents, the predator alone cannot, unfortunately, keep the pests at an acceptable level, so we feel that applying chemical pesticides is inevitable.

Proteus® is a new, highly effective contact and systemic insecticide providing knockdown and residual control against thrips in onion crops. Proteus also provides control against aphids, potato tuber moths, and the potato/tomato psyllids in potato crops. Proteus contains a new explored formulation, an oil-based adjuvant, that assists the coverage, retention, and penetration of the product to the leaves, thus providing access to those areas where thrips commonly reside. Proteus exhibits both systemic and contact effects, allowing its active ingredients to protect new areas in the plant. The insecticide Proteus is a combination of thiacloprid and deltamethrin (Bayer Crop Science 2012).

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Deltamethrin is a synthetic pyrethroid pesticide that kills insects through contact and through stomach action. It is applied for a range of commercial crops. Deltamethrin is based structurally on natural pyrethrins, which rapidly paralyze the insect's nervous system giving a quick knockdown effect (Haug and Hoffman 1990).

Pirimicarb is a systemic insecticide with contact and stomach action, and cholinesteral inhibitors used as a selective aphicide. It is known as an environmentally low risk carbamate compound and it is known for having a low toxicity to natural enemies (Loginova 1984).

Pymetrozine is a pyridine azomethine, systemic contact compound, used for controlling sucking pests like aphids and whitefly on various crops. This insecticide disables the salivary pump of sucking pests which stops the insects from feeding. This compound has a low toxicity to mammals and is safe for birds and non-target arthropods, making it appropriate for IPM programs (EPA 2000).

Inappropriate use of chemicals can decrease the efficiency of natural enemies, hence, some tests are necessary to minimize the chemicals' adverse effects. Bakker *et al*. (1992) believe methods for such tests should be rapid, low cost, repeatable, controllable, efficient, and be able to reliably predict the effects of pesticides in field conditions so as to prevent the high costs of additional tests.

The development of methods and techniques used to test the side effects of chemicals on beneficial organisms began in Europe. Nowadays, there is increasing woldwide public attention focused on this subject (Haskell 1998). Several organizations are active in this field but the primary focus is on the beneficial insects applied by the International Organization of Biological Control (IOBC). This organization is active in identifying pesticides compatible with biological control. Various methods have been developed to test the toxicity of pesticides on beneficial insects. Some of the testing is designed by the IOBC (Hassan 1992). Modern standard bioassay tests evaluate the overall effects of pesticides on pests and natural enemies, evaluating both the lethal and sub-lethal effects.

In general, the three types of tests recommended by the organization and used in most countries are the laboratory, semi-field, and field tests. Following, is a summary of studies conducted by some researchers: Lucas *et al*. (2004) studied the effects of some insecticides on the lady beetle *Coleomegill amaculata* De Geer in the laboratory. Their results showed that imidacloprid has a high toxicity for both adults and larval stages.

Bozsik (2006) studied the effect of several pesticides, including imidacloprid and deltamethrin on the lady beetle *Coccinella septempunctata* L. in the laboratory. In his study, he found imidacloprid and deltamethrin to be relatively harmful insecticides for the predator.

The effect of the insecticides carbofuran and imidacloprid on *Hippodamia undecimnotata* (Schneider) was studied by Papachristos and Milonas (2008) in Greece. This study showed that carbofuran and imidacloprid lead to a 67.6 and 52.2% survival of the predator, respectively. Imidacloprid is also toxic for the larvae and adult of lady beetle *C. septempunctata* (Mizell and Sconyers 1992). Pirimicarb is considered as a low-risk pesticide for predators such as the green chrysopid (Badawy and Arnaouty1999).

In another study, immature stages of the parasitoid *Lysiphlebus fabarum* (Marshall) were exposed by dipping mummified aphids into insecticide solutions. Abamectin, imidacloprid, and pymetrozine caused 44.8, 58.5, and 14.5% mortality of mummies, respectively (Sabahi *et al*. 2011).

In this study, the toxicity of Proteus, pymetrozine, deltamethrin, and pirimicarb were tested on *H. variegata* to provide a background for implementation of integrated aphid management programs.

MATERIALS AND METHODS

Rearing

Broad bean *Vicia faba* L. cultivar *serakhsi* was planted in plastic pots containing sawdust. The seeds were treated with the fungicides benomyl and mancozeb, before planting at a depth of 3 cm. Irrigation was done every day and fertilization was done with Horti® fertilizer (2% solution in water) every 4 days. When the plants reached the 5–6 leaf stage, they were transferred to a growth chamber where the temperature was 25±1°C, 65±10% relative humidity (RH), and there was a photo period of 16:8 (L:D). Black bean aphid *Aphis fabae* Scopoli collected from the field were transferred, using a soft brush, to the plant leaf surface. After establishment of a colony, several young plants were introduced every three days to keep the colony healthy.

Variegated adult lady beetles *H. variegata* were collected from an alfalfa field on the Agricultural Campus, Karaj. They were transferred to plastic chambers (15x20 and 12 cm deep), and covered with 200 mesh organdy. Pieces of paper and leaves were used as oviposition bedding for the predator for protecting the eggs. A piece of moist cotton provided moisture in the chamber. After hatching eggs, each larva was introduced to a 9 cm plastic Petri dish containing aphids. Honeydew collected from infected plants was also used to feed the predator.

Insecticides

Insecticides used in this study were Proteus OD 110, a mixed insecticide including thiacloprid 100 g active substance (a.s.)/l and deltamethrin 10 g a.s./l, (Bayer Agricultural Products, Germany), pymetrozine WP 25 g a.s./kg (Syngenta Crop Protection, Switzerland), deltamethrin EC 25 g a.s./l (Bayer Agricultural Products, Germany), and pirimicarb WP 50 g a.s. /l (Gyah Corporation, Iran).

The recommended concentration (a.s.) of each insecticide in tap water was used to evaluate the acute toxicity within 24 h to L_3, L_4, and adults of *H. variegata* (Proteus 250 ppm, pymetrozine 625 ppm, deltamethrin 312 ppm, and pirimicarb 250 ppm). The control was treated with tap water. In another test, preliminary bracketing was done to determine that the concentrate caused about 10 to 90% mortality. Finally, five doses of each insecticide were used for adult exposure. A potter tower Burkard® with a delivery rate of 1.5±0.01 mg/cm^2 at 15 PSI pressure, was used for treating the glass plates (10x10 cm) which formed the bottom of the exposure cages. Treated plates were allowed to dry for 1 hour. Plastic glass rings (6 cm in diameter and 3 cm in height) containing ventilation windows, with another untreated glass plate, completed the cage. A piece of moist cotton was used as a water source. Fifteen individuals were introduced into each cage. The cages were transferred to the growth chamber. The growth chamber had the above mentioned conditions. Mortality was assessed after 24 hours.

Insecticides were classified into IOBC categories, according to how harmful the insecticides were. Insecticides with a mortality less than 30% were classified as category 1 (harmless), a mortality between 30 to 79% were classified as category 2 (slightly harmful), between 80 to 99% – category 3 (moderately harmful), and insecticides with a mortality over 99% were classified as category 4 (Harmful) (Sterk *et al.* 1999).

Statistical analysis

A completely randomized design (CRD) with four replications was used as the experimental design. For statistical analysis, SAS software was used (SAS Institute 2001). The means were separated by the least significant difference (LSD) test ($p = 0.01$). The Polo Plus (LeOraSoftware 2006) was used to estimate lethal concentrations, their 95% confidence limits as well as other parameters.

RESULTS AND DISCUSSION

Acute toxicity of field recommended concentrations

Mortalities of treated *H. variegata* from the field recommended concentrations of various insecticides were significantly different ($F = 241.67$; $df = 11, 47$; $p < 0.01$) (Table 1). Proteus showed strong toxicity toward the different life stages of the predator. However, the field recommended concentrations of pymetrozine and pirimicarb caused less than 50% mortality.

Based on IOBC classification, both of the insecticides pirimicarb and pymetrozine are classified as having a Class 1 (harmless) level of toxity, deltamethrin is classified as having a Class 2 (slightly harmful) level of toxity, and Proteus as Class 4 (harmful).

LC_{50} and LC_{30} of Insecticides

The LC_{50} and LC_{30} values of four insecticides with 95% FL were obtained for adult *H. variegata*, 24 h after treatment (Table 2). Based on the lethal concentration (LC_{50}) values 24 h after treatment, adult *H. variegata* were most susceptible to Proteus, followed by deltamethrin, pymetrozine, and pirimicarb values of 35.977, 358.757, 915.667, 2616.113. Based on the sublethal concentration (LC_{30}), these values were 22.718, 261.957, 569.879, and 1521.424 (Table 2).

The linear regression test of parallelism by PoloPlus software, showed that the hypothesis of parallelism was not rejected and the lines had slopes that were not significantly different ($X^2 = 6.53$, $df = 3$, tail $p = 0.088$, $p > 0.05$). However, the hypothesis of equality (equal slopes, equal intercepts) was rejected ($X^2 = 150$, $df = 6$, tail $p \cong 0.000$, $p < 0.05$).

Research has shown that the only way to a successful and sustainable control of pests is through the use of IPM programs. In an IPM program more emphasis is put on chemical and biological control. Each method alone has its own limits and cannot decide the aims of the program. In this framework, only those insecticides which reach the maximum selectivity for biological agents and which show the minimal adverse effects on natural enemies should be used (Bartlett 1966; Croft and Brown 1975).

This research utilized the standard bioassay tests based on the recommended methods of the IOBC. We tried to expose the various life stages of cocinellid *H. variegata* to some insecticides including: Proteus, pymetrozine, deltametrine and pirimicarb. The reason for the exposure was to determine the effects of the pesti-

Table 1. Mortality rates of the predator *H. variegata* treated with recommended dose of each insecticide and classified according to IOBC evaluation categories

Insecticide	Dose [ppm]	Life stage	Mortality % (±SE)	Classification[1]
Proteus	2,500	L_3	100.00±0.00 a	4
		L_4	100.00±0.00 a	4
		adult	100.00±0.00 a	4
Deltamethrin	1,250	L_3	60.00±2.72 b	2
		L_4	53.33±2.72 c	2
		adult	38.33±3.19 d	2
Pymetrozine	2,500	L_3	40.00±2.72 d	2
		L_4	15.00±1.67 fg	1
		adult	10.00±1.92 gh	1
Pirimicarb	500	L_3	21.67±3.19 e	1
		L_4	20.00±2.72 ef	1
		adult	6.67±2.72 h	1

Values followed by the same letter are not significantly different from each other according to LSD ($p < 0.01$);
1 – harmless, 2 – slightly harmful, 4 – harmful; SE – Standard Error; [1] International Organization for Biological Control

Table 2. Toxicity of different insecticides to the adults of *H. variegata*

Insecticide	N	Slope ±SE	LC_{30} [ppm] (95% CI)	LC_{50} [ppm] (95% CI)
Proteus	270	2.627±0.436	227.181 (162.446–280.697)	359.766 (293.029–435.015)
Deltamethrin	270	3.840±0.546	1047.829 (857.380–1200.732)	1435.030 (1260.157–1615.523)
Pymetrozine	270	2.546±0.399	2279.517 (1622.252–2804.685)	3662.67 (3128.956–4188.173)
Pirimicarb	270	2.228±0.388	3042.848 (1900.654–3966.629)	5232.225 (4023.903–6420.879)

N – number of specimens; CI – Confidence Interval; SE – Standard Error

cides on the predator, and to provide the context for the use of selective insecticides. *H. variegata* is a voracious predator with a high reproductive rate and therefore is able to strongly suppress aphid populations.

This study showed that the predator is very sensitive to the insecticide Proteus. This result is consistent with the findings of Lucas *et al*. (2004) who tested several insecticides on Lady beetles *C. maculata* under laboratory conditions. Their results showed that imidacloprid is highly toxic to both the adult and larval stages. Likewise. Huerta *et al*. (2003) tested imidacloprid on the third instar larvae of green lacewing *Chrysoperla carnea* and found the insecticide to be highly toxic, causing high mortality in the predator.

Neonicotinoid compounds can affect arthropod natural enemies when they are feeding on pollen, plant tissue, plant sap or prey treated with insecticides. This exposure can lead to the death of the agents. Mizell and Sconyers (1992) believe that imidacloprid is toxic for both larvae and adults of cocinellids. Death caused by Proteus can be related to the knock-down effect, and anti-nutrition of its components. Thiacloprid (one of Proteus components) can impact the bug by interfering with the transmission of nerve messages. Deltamethrin causes paralysis by blocking the Na channel in the nerve cells of the predator, and causes rapid death.

As far as toxicity goes, we placed deltamethrin in category II (slightly harmful), which is in agreement with the findings of Bozsik (2006) who tested the effect of some insecticides including deltamethrin, on the ladybug *C. septempunctata*, under laboratory conditions, and described the insecticide as moderately harmful for the predator. These results also revealed the low susceptibility of the predatory coccinellid to insecticides: pirimicarb and pymetrozine. The findings are consistent with the findings of Badawy and Arnaouty (1999) who described pirimicarb as a low-risk insecticide for the green lacewing predator.

Pymetrozine had less than a 20% mortality for *O. tristicolor* in a laboratory evaluation, and was described as safe by James (2004). Also, Jansen *et al*. (2011) reported the side effects of pymetrozine on five natural enemy species of aphids, including coccinellid *Adalia bipunctata*. These scientists expressed the high selectivity of the insecticide.

This study showed that the highest rate of mortality of *H. variegata* happened when this predator was treated with proteus. This fact meant that the insecticide was given a category 4 level of toxicity or harmfulness. For these reasons, the test should be continued in semi-field and field conditions.

According to the IOBC rating, deltamethrin is placed in category 2 level of toxicity, which can be interpreted as moderately harmful. It means the deltamethrin can be used as part of integrated control of *A. fabae*. For more precise results, semi field and field tests are recommended.

The lowest rate of mortality in larvae and adults of predators occurred in the pirimicarb and pymetrozine treatments which showed no statistical difference. This low rate was related to the selectivity characters of both insecticides. The results enabled them to be placed in category 1 with a toxicity level of harmless. Since these insecticides are category 1, it means they can be used as a part of an aphid IPM program alongside the predator. According to the IOBC, since these insecticides were harmless in the testing done in laboratory conditions, no further studies were required in semi-field or field conditions. Both of these compounds are systemic and prevalent in aphid control.

Linear tests of equality and parallelism may be the most efficient methods for comparing probit regressions. When lines are parallel but not equal, as we observed in this study, their slopes are not significantly different, even though their intercepts differ significantly. Biologically, the slope of a probit regression estimates the change in activity per unit change in concentration. Pharmacological evidence described by some researchers, suggests that the dose-response slope of a probit regression can also reflect the quality of enzymes involved in detoxification (Robertson *et al*. 2007).

Due to an increasing use of natural enemies alongside the use of pesticides, it is important to consider the effects of pesticides when taking into account beneficial protection. So the success of the biological control in an IPM program largely depends on the selectivity characters of the chemicals used. By choosing low-risk pesticides for beneficial organisms, the role of the natural enemies can be reduced and we can reach a more successful pest control.

The use of compounds with high selectivity characters, and reducing the use or withdrawing hazardous compounds, and decreasing the frequency of applications as well as making sure to employ chemicals in low concentrations are simple methods for conserving beneficial insects. These methods not only protect the natural enemies as valuable biological resources, but can also help ease environmental pollution. These are the practical steps for the further development of integrated pest management programs.

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